

Response of the Pituitary and Thyroid to Tropic Hormones in Sprague-Dawley versus Fischer 344 Male Rats

P. A. Fail,*¹ S. A. Anderson,* and M. A. Friedman†

*Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709-2194; and †Cytec Industries, Inc., 5 Garrett Mountain Plaza, West Paterson, New Jersey

Received August 20, 1998; accepted January 4, 1999

Modulation of endocrine function is frequently a confounding factor in the interpretation of chronic rodent toxicology studies. Of particular interest are agents that cause deviation of thyroid hormone homeostasis and result in thyroid cancer for rodents. An endocrine challenge test (ECT), commonly used to study endocrine organ health in human and veterinary medicine, quantifies the response of the thyroid to tropic hormones. This study compared the response of Fischer (F344) and Sprague-Dawley (SD) rats to a thyrotropin-releasing hormone (TRH) ECT and a thyroid-stimulating hormone (TSH) ECT and characterized the dose-response curve. TSH, thyroxine (T4), triiodothyronine (T3), and prolactin responses were characterized for several doses of TRH over a 4-h time period. Animals were equipped with intra-atrial cannulae and were free moving at all times during blood sampling. Both strains of rats responded to intravenous TRH by releasing TSH into their blood in a dose-responsive fashion. At doses of ≥ 100 ng, TSH concentrations were increased by more than 2-fold at 2 min. Concentrations reached a maximum at 15 min for doses of 100 ng/100 g body weight (bw) to 5000 ng/100g bw. The effective dose 50 (ED_{50}) of TRH (that dose causing release of half maximal TSH concentrations) was 61 ng in F344 rats and 78 ng in SD rats. The ED_{75} was 173 ng and 217 ng/100 g bw, respectively. The response of T4 and T3 after TRH ECT and TSH ECT was highly variable. F344 rats responded with an increase in levels of both hormones, starting at 60 min and continuing through 240 min. In SD rats, the presence of a thyroid hormone response (T4) was present, although that of T3 was not clear. These data provide essential information for design of toxicology studies focused on the effects of toxicants and drugs on the pituitary-thyroid axis.

Key Words: pituitary; thyroid; thyrotropin-releasing hormone; thyroid-stimulating hormone; endocrine challenge test

Homeostatic control mechanisms of thyroid gland function in humans have been well established. Pulsatile secretion of thyrotropin-releasing hormone (TRH) from the median eminence of the hypothalamus stimulates thyroid-stimulating hormone (TSH) secretion from the anterior pituitary thyrotrophs. TSH stimulates the synthesis and secretion of triiodothyronine

(T3) and thyroxine (T4) from the thyroid gland, as well as thyroid gland growth. T4 is converted to T3 by the liver, kidney, muscle, and the anterior pituitary, and both exert direct negative feedback on TRH and TSH secretion (Williams, 1994). Disruption of these relationships can lead to altered metabolism, benign or cancerous tumors, and eventual loss of life.

Disruption of the endocrine homeostatic controllers can result in the development of mammary tumors (Meites *et al.*, 1971; Welsch *et al.*, 1970), pituitary tumors (Brown *et al.*, 1979; Buckingham and Gillies, 1990), interstitial cell tumors, and thyroid tumors in rodents (Hill *et al.*, 1989; McClain, 1992), and endometrial carcinomas in rats (Neumann, 1991). These tumors can be induced by excessive tropic hormone exposure over a long period of time. Hormonal hyperstimulation is followed by cellular hyperplasia in a dose-dependent manner. Tumor induction by this mechanism can be predicted far in advance by increased hormone concentrations in serum or by measuring the hyperplastic response. Drugs or environmental chemicals may also cause tumorigenesis by overstimulation or inhibition of the tropic hormone. Thus, endocrinopathies present in short-term rodent studies may be predictive of tumor induction in long-term studies. For example, altered feedback mechanisms, secretory rates, or patterns may be predictive of hypertrophy and hyperplasia that would precipitate conditions in the thyroid favorable for neoplasia.

Based upon this concept, chemicals that cause an imbalance in the hypothalamic-hypophyseal (pituitary) thyroid axis (HHTA), would be expected to induce thyroid tumors. In many cases, elevated TSH is associated with thyroid tumors in humans, although it is not likely the mechanism involved in causation of human thyroid neoplasms. Consequently, TSH and thyroglobulin concentrations are used to diagnose human thyroid follicular neoplasia. Thyroglobulin is specifically produced by the follicular cell (Hennen, 1994), and its presence in abnormal concentrations in human blood is used to monitor the spread of thyroid cancer (Sheppard, 1986).

In contrast to humans, TSH stimulation is suspected as the cause of thyroid neoplasms (McClain, 1992) in rats and other rodents. There are significant rodent/human differences in thy-

¹ To whom correspondence should be addressed. Fax: (919) 541-7208. E-mail: patf@rti.org.

roid physiology that could explain this observation (McClain, 1992), including the absence of thyroglobulin and the shorter half-life of T3 and TSH in rodents. In addition, the incidence of spontaneous thyroid neoplasia is 500 times higher in rodents. The endocrine response to increased TSH secretion in rodents is unclear. It is not known whether the hormone feedback mechanisms and the secretory patterns of these hormones are similar in rodents and humans.

Endocrine-mediated toxicity and testing to detect the disruption of normal hormonally controlled processes is currently the focus of several national committees. An endocrine challenge test (ECT) would quickly test endocrine organ health during exposure to a drug or a potentially toxic chemical. For example, a TRH ECT could be used to test the pituitary's response to TRH (normal release of TSH), and the TSH ECT could be used to test the thyroid's response to TSH (normal release of T3 and T4).

The objectives of this study were (1) characterization of hormonal response to TRH; (2) determining dose-response curves for TSH, T3, T4, and prolactin in rats; (3) characterization of the TSH stimulated dose-response curve for T3 and T4 in male rats; and (4) to compare these responses in two strains commonly used in toxicology studies—Sprague-Dawley (SD) and Fischer 344 (F344) rats.

METHODS

Animals and Animal Husbandry

Thirty adult male SD rats (one shipment, Group A) and 65 adult male F344 rats (two shipments, Groups B and C) were received for use in the TRH and TSH challenge experiments (Experiments 1 and 2, respectively). The age of the rats at the beginning of the experiment was 9–11 weeks. All rats were individually identified by ear tag after arrival. During a 7-day quarantine period, animals were randomly assigned singly to solid-bottom, polycarbonate cages ($5 \times 11\frac{1}{2} \times 7$ in.) with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ). Feed (#5002 Purina Certified Rodent Chow) and deionized/filtered tap water in polycarbonate bottles with stainless steel sipper tubes were available *ad libitum* throughout the study. The animal room used for this study was maintained on a 14:10-h light:dark cycle. Target conditions for temperature and humidity in the animal rooms were maintained at 68–75°F and 40–70%, respectively. Body weights were recorded once during surgery and at each challenge. Fourteen animals died due to surgical complications.

Cannulation Surgery

Animals were fitted with indwelling jugular cannulae using the Preclinical Mini-Port™ (SIMS-Deltec, St. Paul, MN), with the intent of having at least five animals per challenge group patent for each blood sampling. The Preclinical Mini-Port™ is an implantable, vascular access device designed to permit repeated access for intravenous or intra-arterial injection/infusion. The miniport device consists of a polysulfone/titanium portal, with a self-sealing silicone septum and a preattached radiopaque polyurethane catheter with two moveable suture beads. Animals were anesthetized with a combination of ketamine, xylazine, and acepromazine (10:1:0.1; 80–100 mg/kg body weight) (Butler, Kernersville, NC). After shaving and scrubbing the area, a transverse incision was made just caudal to the scapulae. A subcutaneous pocket large enough to hold the portal was made. A ventral incision was made from below the point of the jaw to just above the clavicle to allow externalization of the right jugular vein. The attached catheter was placed through a subcutaneous

tunnel from the portal site out the ventral incision. To insert the catheter, a hole was made in the right external jugular vein using a 20-gauge needle. The catheter was then grasped with small forceps and the tip inserted through the hole. The cannula was advanced until the tip reached the right atrium of the heart (the distance was body weight-dependent). The catheter was then sutured in place. A tension loop was formed in the cannula under the skin at the ventral incision. The port was sutured in place and all incisions closed.

The port was accessed through the skin using a 24-gauge, noncoring, nonsiliconized needle and a 5-ml syringe. The system, flushed with sterile saline (0.85%) and heparinized saline (100 IU heparin/ml saline) (Butler, Kernersville, NC), was used to lock the system. The miniport was flushed daily with sterile saline for the first postoperative week. It was then flushed twice weekly throughout the rest of the study. Animals were given 10–13 days to recuperate before administration of the first ECT.

Test Chemical Dosage Preparation and Stability

TRH challenge (Experiment 1). TRH, a synthetic tripeptide from Sigma Chemical Company (St. Louis, MO), is highly stable in lyophilized form. Acetate salt (84% [Lot No. 40H5830] and 79% [Lot No. 33H5820] peptide content) was used. The TRH challenge concentrations were mixed in 0.85% saline at 0, 10, 25, 100, 250, 1000, 2500, and 5000 ng/0.1 ml, and rats were dosed once at a volume of 0.1 ml/100 g body weight (1 ml/kg).

TSH challenge (Experiment 2). The TSH was kindly provided by the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the Department of Agriculture (Lot No. NIDDK-rTSH-I-9, AFP-11542B). The TSH was mixed in 0.85% saline at 200, 400, 800, and 1600 ng/0.1 ml, and rats were dosed once with 0.1 ml/100 g body weight (1 ml/kg).

Experimental Design

Experiment 1 evaluated thyroid and pituitary response to TRH doses, whereas Experiment 2 tested thyroid responses to various doses of TSH. Designs of Experiments 1 and 2 are detailed in Tables 1 and 2, respectively.

These experiments were conducted according to the following regimen. For Experiment 1 (TRH ECT; Table 1) the experiment was repeated (replicated) within strain and group. Two replicates were conducted within Group A (using the same SD males twice), three replicates were conducted within Group B, and three replicates were conducted using the Group C animals. Use of the within-group replicates was considered legitimate; that is, animals could be used repeatedly at intervals of greater than 5 days, as endocrine effects following each ECT dose were not expected to last for more than 24 h. Animals were given 8–26 days of rest between sampling periods to ensure that hematocrits remained within 10% of the baseline hematocrit. Animals were assigned to treatment groups in such a way that an animal that served as a control in one instance served in a different treatment group during the next replicate of the experiment. An individual animal was never given the same dose twice and animals within dose groups were each assigned to different dose groups for the next replicate. Data were pooled (within strain and experiment) after statistical analyses determined that no differences existed due to replicate group. Experiment 2 (TSH ECT; Table 2) was done in one replicate for each strain of rat, with animals assigned at random to treatment groups.

Cannula Patency

For these experiments, animals were considered patent only if blood samples could be withdrawn via the cannula. Animals for which only infusion was possible were not considered patent. The F344 rats proved to have a lower initial patency rate (Table 3) and were more difficult to prepare for endocrine experiments because of their smaller size and inconsistent response to anesthesia (Group B). Thus, a second group of F344 rats was needed (Group C). F344 rats were more sensitive to the amount of heparin in the saline used to

TABLE 1

Experimental Design, Experiment 1: Endocrine Response to TRH—A Validation for Dose Response; Secretion of TSH, T3, T4, and Prolactin in Sprague-Dawley and Fischer 344 Male Rats

Treatment group	Dose (ng/100 g) ^a	Vehicle	Sprague-Dawley ^b	Fisher 344 ^c	
			Group A	Group B	Group C
Vehicle	0	Saline	4	7	9
TRH	10	Saline	6	0	4
TRH	25	Saline	8	5	0
TRH	100	Saline	7	7	8
TRH	250	Saline	8	6	8
TRH	1000	Saline	5	7	9
TRH	2500	Saline	0	0	9
TRH	5000	Saline	0	0	7

^a Doses were administered once by injection through jugular cannula.

^b For the Sprague-Dawley animals, the TRH ECT was conducted in two replicates. Animals still patent after replicate 1 were reassigned to a different dose group for replicate 2.

^c The TRH ECT in F344 rats was conducted in six replicates (three per group). Animals remaining patent after a given replicate were reassigned to dose groups for the next replicate. Because of poor patency with group B, a second group of animals was cannulated (group C) to increase the *n*.

flush their cannulae. (Many had poor recoveries and more bleeding than SD rats). The patency rate improved with the second group of F344 rats (Group C). Surgical success and patency information are summarized in Table 3.

With technical experience, a higher patency rate was possible, with over 50% of the animals initially patent, and remaining patent for nearly 2 months in Group C. This difference may be due in part to a slower growth rate in the Group C (F344) animals.

Blood Sampling Regimen

For serial sampling, the rats were fitted with specially designed jackets that allowed access to the port. A tethering system was attached to allow blood sampling without handling the rat. This consisted of a spring tether, swivel, and polyurethane tubing with a 1-cc syringe attached. A specially designed, non-coring needle was attached to the tubing and opposite end of the tether, inserted into the port, and fastened to the rat jacket with Velcro. The system was completely flushed with heparinized saline after completion of each ECT and before the jacket and tether were removed. With this design, the rat was allowed to remain unrestrained and untethered when sampling was not occurring. During the sampling periods, rats were able to move freely about their cages attached to the tethering system. Prior to the first ECT, all rats were acclimated to their jackets by wearing them daily for 3 h/day for 7–10 days. Animals were jacketed at 30 min (SD) or 60 min (F344) before the first sample of each series was collected.

Blood samples (0.6 ml each) were drawn at –30, –15, and 0 min to determine

baseline estimates for TSH, T3, T4, and prolactin. The challenge dose was then administered, and samples were collected at 2, 15, 30, 45, 60, 120, 180, and 240 min following infusion of the TRH. Time points for sample collection in the TSH ECT were –30, 0, 30, 60, 120, and 240 min postchallenge in SD rats, and the same time points as after TRH for the F344 rats. Blood samples were placed in 1.5-ml heparinized polypropylene-capped tubes, centrifuged at 6000 rpm for 2 min; plasma was collected and frozen at –20°C until analysis. Red blood cells were resuspended in heparinized sterile saline and reinfused into the rat of origin following collection of the next blood sample. In this way, total blood volume and hematocrit were maintained during the sampling period.

Endocrine Measurements

Hormones T3, T4, TSH, and prolactin were determined in each blood sample when sufficient plasma was available. Hormone concentrations were determined in single aliquots using radioimmunoassays (RIA) validated for male SD and F344 rats in our laboratory. Blood samples were drawn through cannula via syringes, placed into heparinized tubes, and centrifuged at 6000 rpm for 2 min to collect plasma.

The T4 was determined by a solid-phase ¹²⁵I RIA designed for the quantitative measurement of total circulating T4 in plasma. The T4 RIA kit, utilizing T4 antibody-coated tubes and ¹²⁵I-labeled T4, was purchased from Diagnostic Products Corporation (DPC; Los Angeles, CA). The T4 standard was purchased from Sigma Chemical Company (St. Louis, MO), and the curve was

TABLE 2

Experimental Design, Experiment 2: Endocrine Response to TSH—A Validation for Dose Response; Secretion of T3, T4, and Prolactin in Sprague-Dawley and Fischer 344 Male Rats

Treatment group	Dose (ng/100 g) ^a	Vehicle	Sprague-Dawley (Group A)	Fischer 344 (Group C)
Vehicle	0	Saline	3	6
TSH	200	Saline	2	0
TSH	400	Saline	3	5
TSH	800	Saline	3	0
TSH	1600	Saline	0	7

^a Doses were administered once by injection through jugular cannula.

TABLE 3
Comparison of Patency Rates and Mortality/Morbidity in Male Sprague-Dawley and Fisher 344 Rats

	Group A (SD)	Group B (F344) ^a	Group C (F344)
Animal preparation and recovery			
Number purchased	30	30	35
Surgical/anesthesia deaths	4	5	5
Jugular damage (not used)	0	3	2
Additional never patent (from surgery)	3	7	6
Total initially patent (at first challenge)	23	15	22
Patency rates ^b			
9–13 days	23/23 (100%)	15/15 (100%)	—
13–17 days	21/23 (91%)	13/15 (87%)	22/22 (100%)
20–22 days	18/23 (78%)	11/15 (73%)	20/22 (91%)
27–31 days	16/23 (70%)	7/15 (47%)	19/22 (86%)
41–43 days	13/23 (57%) ^c	—	16/22 (73%)
50–56 days	—	—	16/22 (73%)
71–85 days	—	—	6/22 (27%)
Mean length of patency (days)	32.91 ± 2.65	23.40 ± 1.96	52.18 ± 4.39
Clinical problems			
Exteriorized port	—	1	5
Hematomas at site of port	—	9	1

^aDue to lack of experience with F344 rats, patency rates were not adequate. A second set of F344 rats was cannulated (group C).

^bDays are postsurgery in those initially patent for sampling. Not all patent animals were challenged at each sampling time.

^cTwo animals remained patent 128 days, one for 190 days, and one for greater than 256 days.

prepared in 0.01M phosphate-buffered saline containing 1% BSA and 0.1% sodium azide, pH 7.6.

The T3 was determined by a solid-phase ¹²⁵I RIA designed for the quantitative measurement of total circulating T3 in plasma. The T3 RIA kit utilized T3 antibody-coated tubes and ¹²⁵I-labeled T3 (DPC, Los Angeles, CA). The T3 standard (Sigma Chemical Co., St. Louis, MO) curve was prepared in a T3-free rat plasma matrix prepared at Research Triangle Institute by charcoal stripping normal, strain-specific SD plasma.

Both assays were validated using added mass of 80–320 ng/dl in T3/T4-free rat plasma. Intra-assay variation for T3 and T4 RIAs was 2–12% for mass added, and interassay variation was 7–20% for mass added. Recovery of added mass was excellent (93–126%). Index of parallelism was ~ 107% for T3 and ~ 118% for T4. Assay sensitivity was 6.25 pg for T3 and 0.16 ng for T4.

The TSH RIA was determined by a double antibody miniassay designed to use 50 µl plasma or less per determination. The TSH kit contained an antiserum to rat TSH (anti-rat TSH-S-6, AFP329691Rb), hormone for iodination (rat TSH-I-9, AFP-11542B), and a rat TSH standard (rat TSH-RP-3, AFP-5512B) kindly provided by the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the Department of Agriculture. The ¹²⁵I-labeled TSH was purchased from Corning Hazleton, Inc. (Vienna, VA), and the second antibody was a goat anti-rabbit immunoglobulin purchased from Sigma Chemical Co. (St. Louis, MO). Intra-assay variation ranged from 3.5–11.6% for mass added at 2, 10, and 50 ng/ml, and interassay variation was 0.4–29% at the same mass. Recovery of added mass was 87–112%, and the index of parallelism was 108%. Assay sensitivity was 20.5 pg.

The prolactin RIA was determined by a double antibody RIA employing a polyethylene glycol (PEG) second antibody-precipitating solution to accelerate the separation of bound and free hormones. The prolactin kit contained an antiserum to rat prolactin (anti-rat PRL-S-9, AFP-131581570), hormone for iodination (rat PRL-I-6, AFP-10505B), and a rat prolactin standard (rat PRL-RP-3, AFP-4459B) kindly provided by the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the

Department of Agriculture. The ¹²⁵I-labeled prolactin was purchased from Corning Hazleton, Inc. (Vienna, VA), and the second antibody (goat anti-rabbit immunoglobulin) was purchased from Sigma Chemical Co. (St. Louis, MO). The prolactin RIA was characterized by an intra-assay variation of 3.4–17.7% for 0–100 ng/ml (added mass) and an interassay variation of 5.8–29%. Percent recovery of added mass ranged from 68–141%. The index of parallelism was 105%. Assay sensitivity was 10 pg.

Statistics and Data Presentation

Parametric statistical procedures were applied to parametric data from this study. Appropriate General Linear Models (GLM) procedures (SAS Institute, Inc., 1989a,b, 1990a,b,c) for the Analyses of Variance (ANOVA) were employed. Prior to GLM analysis, a log₁₀ or square root transformation was performed on hormone data where needed to satisfy the assumptions of the ANOVA (Snedecor and Cochran, 1967). If transformation data were not homogeneous, the nonparametric statistics were used. Bartlett's test for homogeneity of variance (α level = 0.05) was performed on all data prior to ANOVA analysis. GLM analysis with repeated measures ANOVA for parametric data was used to determine whether a significant challenge dose effect had occurred for selected measures. LSMEANS (*t*-test) for unbalanced data or Mann-Whitney U (for nonparametric data) was used to compare each chemical exposed group to the vehicle control group for a specific time period. A two-tailed test was used for all pairwise comparisons. In addition, data were also converted to percent change from the time zero value. These data are presented for T3, T4, and prolactin. The ED₅₀ and ED₇₅ of TRH were estimated using nonlinear regression analysis (SigmaPlot[®] 4.0) for plasma TSH, after TRH ECT in the SD and F344 rats. Plasma TSH values from all dose groups at the 15-min time point were used. Equations used were (1) SD rats, three-parameter hyperbola ($f(x) = y_0 + (ax/b + x)$), and (2) F344 rats, three-parameter hyperbola ($f(x) = ax/b + x$). Parameter estimates were computed by SigmaPlot[®]. ANOVA *p*-values were < 0.05 for both regressions (0.0012 and < 0.0001). Regression equations were then solved for *x* to get the ED₅₀ and ED₇₅ estimates. TSH response values used were 8.5 and 12.75 for SD rats and 6.25 and 9.37 for F344 rats (ED₅₀ and ED₇₅ calculations, respectively).

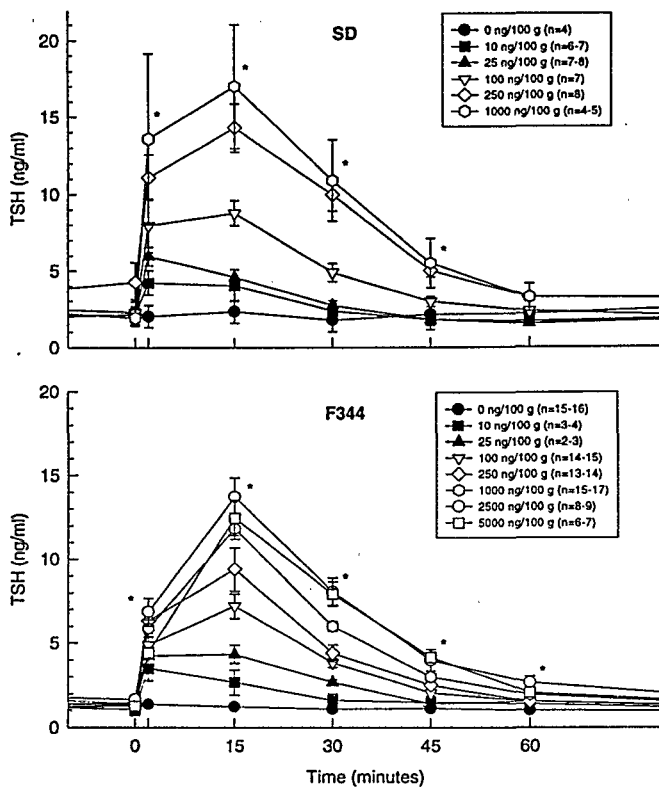


FIG. 1. Plasma TSH after TRH challenge in adult male SD and F344 Rats. For TSH in both strains of rat, values ranged from 1.5 to 17 ng/ml. The standard error of the mean ranged from 0.20 ng/mL to 5.44 ng/mL, as high as 40% of the mean in prechallenge samples and as low as 10% of the mean in postchallenge samples for all time points across all dose groups in the SD rats. In F344 rats, SEM values were between 0.20 and 1.28 ng/mL; means ranged from 0.60 to 13.71 ng/mL. Repeated measures ANOVA detected a significance due to effect of time, dose, and a time by dose interaction for both strains of rat. *Significant different among dose groups within a given time point on ANOVA; $p < 0.05$) Specific pairwise differences are discussed in the text.

Due to the volume of data collected, not all are reported. Significant differences are considered to be those comparisons where p was ≤ 0.05 . Data were selected for each graphic representation of the results to provide a clear understanding of hormonal changes. To do so, standard error bars are not included in percent change data. However, an indication of the overall standard error (SEM) is provided in the figure legends. Statistical significance of the repeated measure analyses is indicated in the text and in the figure legend, and the presence of pairwise significances within time points is indicated by an asterisk. The actual pairwise differences are indicated in the text.

RESULTS

Experiment 1: TRH Endocrine Challenge Test

TSH response to TRH in adult male SD rats. TRH treatment evoked TSH release in a dose-related manner (Fig. 1). Pretreatment baselines were near 2.0 ng/mL in all dose groups except the 250 ng/100 g. Maximal concentrations occurred at 15 min after TRH and average near 15 ng/mL for the two higher dose groups. TSH concentrations were significantly increased

over control concentrations from 2–45 min after 250 and 1000 ng TRH and at 2–30 min after 25 and 100 ng TRH/100 g body weight. The 1000-ng dose was not significantly different from 250-ng dose at any of the time points. The mean TSH concentrations at 2 min were significantly different from controls in all groups except for the 10-ng TRH dose. Average percent responses at 15 min were approximately 100, 200, 300, 350, and 1000% of control for the 10, 25, 50, 100, and 1000 ng/100 g body weight doses.

TSH response to TRH in adult male F344 rats. In F344 rats, TRH treatment evoked TSH release in a dose-related manner (Fig. 1). In most cases, pretreatment baselines were < 2.0 ng/mL in all dose groups. TSH responses to TRH challenge were approximately 2.5- to 9.8-fold baseline values in the seven treatment groups compared to no response for vehicle-treated males. The response to TRH challenge differed between groups ($p < 0.05$; dose by time interaction) in what appeared to be a dose-related increase in maximal response and duration of response. TSH concentrations were significantly increased over control concentrations from 2–30 min after all TRH doses (10–5000 ng TRH/100 g body weight). However, the 1000, 2500, and 5000 ng/100 g body weight doses were not significantly different from each other at any time point. For most of the dose groups, statistically significant differences continued through the 45-min sample. At 60 min, animals given 100 ng/100 g body weight or more still had increased TSH; by 120 min, no differences existed (data not shown). TSH values lower than controls occurred in some of the higher dose groups at 180 and 240 min (data not shown). When TSH data were expressed as percent change from the zero time sample for each animal (i.e., use each animal as its own control), the interpretation of the results changed very little (data not shown). The one exception was an increased length of time that TSH concentrations were significantly increased. That is, a significant response lasted through the 60-min sample for all dose groups.

Thyroid hormone response after TRH ECT in SD rats. Because of tremendous variability in plasma T3 and T4, the percent change in plasma T3 and T4 concentrations of male rats after TRH ECT is presented for selected dose groups to facilitate graphic presentation. TRH treatment and the resultant TSH release did not consistently stimulate T3 or T4 production (Figs. 2 and 3), although T3 increased over 2–4 h in most animals. With the three highest doses of TRH, T3 concentrations were consistently higher than in controls at 1–4 h. Furthermore, the overall mean concentration of T3 was significantly higher after 250 and 1000 ng TRH than after saline (79.10 ± 8.08 and 84.11 ± 10.03 vs. 49.15 ± 6.5 ng/dl for controls).

For T4 (Fig. 3), no consistent pattern was observed, although some dose groups (100 and 1000 ng TRH/100 g body weight) had a significantly higher relative change at 2–4 h after TRH. Time-course plots of TSH, T4, and T3 profiles for two indi-

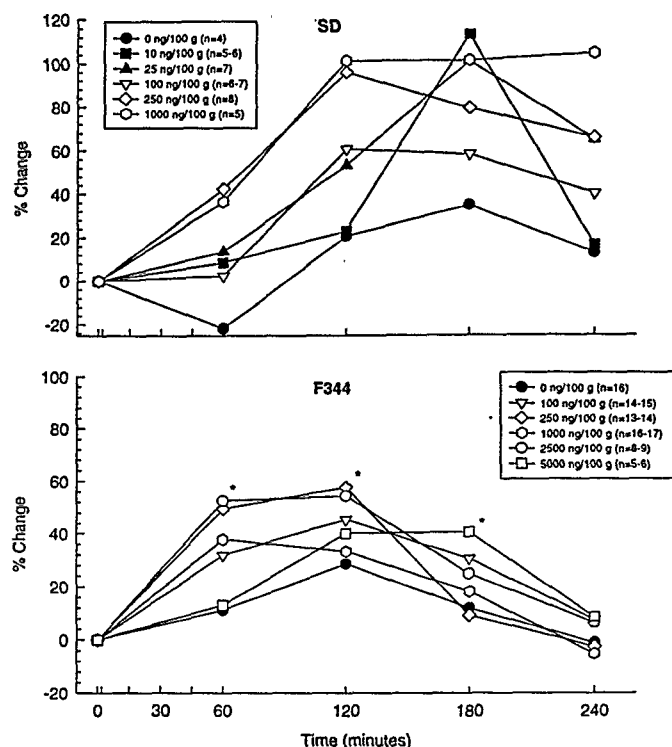


FIG. 2. Percent change in plasma T3 after TRH challenge in adult male SD and F344 rats. For T3 in both strains of rat, the standard error of the mean ranged from 10 to 20% of the mean for all time points across all dose groups. Mean values for individual time points were 22 and 158 ng/ml for both strains, but SD had higher maximum values (raw data not shown). Repeated measures ANOVA detected a significance due to effect of time, dose, and a time by dose interaction for the F344 rats, and an effect of time only for the SD rats. * $p < 0.05$ ANOVA or Mann-Whitney U.

viduals with significant TSH responses (Fig. 4) confirmed that TSH was accompanied by T4 and T3 releases. The T4 release appeared to be a sustained release over the 4-h period, whereas the T3 release after TRH-induced TSH may have been a brief burst (SD1121).

Thyroid hormone response after TRH ECT in F344 rats. In male F344 rats, the plasma T3 and T4 concentrations after TRH were increased (Figs. 2 and 3). The percent change over time (based upon each animal's zero time sample) for T3 and T4 was examined carefully (Figs. 2 and 3). Statistical analysis of mean percent change from time 0 indicated that there was a significant increase of T3 in response to TRH (Fig. 2) at 45 min for the 250-ng and 1000-ng dose groups (data not shown), an increase at 60 min for the 100- to 2500-ng dose groups, and at 120 min for all dose groups ≥ 100 ng TRH/100 g body weight. (Data for 10- and 25-ng dose groups are not shown). The T3 response to TRH had subsided by 240 min at all doses.

The response of T4 in F344 rats to TRH-mediated TSH release appears to be clearer (Fig. 3). It is clear that T4 was increased after TRH in F344 male rats from 2 h up to 4 h at/or above 100 ng TRH. Percent change data (Fig. 3) indicated that

a response after ≥ 100 ng TRH/100 g body weight was clearly evident by 120–180 min, lasting through 240 min. A lower dose (25 ng TRH/100 g body weight) also caused significant changes in plasma T4 at 180 and 240 min after TRH (data not shown).

Plasma T3 and T4 increased simultaneously (following the TSH release) in for individual F344 rats (F344 1179 and F344 1180) (Fig. 5). These responses followed 250 μ g TRH/100 g body weight, reaching maximal response by 120 min for both hormones. The mean percent response at 120 min for this dose group was $33 \pm 6.8\%$ (T4) and $58 \pm 15.9\%$ (T3). Thus, it appears that TRH and the resultant TSH release did stimulate T3 and T4 release from the thyroid of the F344 rats.

Prolactin response to TRH in male SD rats. A TRH-induced spike of prolactin occurred by 2 min after 100, 250, and 1000 ng TRH/100 g body weight. Expressed as percent change, the 100 ng TRH/100 g body weight evoked a significant prolactin increase at 15 min (Fig. 6). Mean prolactin values were statistically different from controls at several other time points, but this did not seem to be treatment related, as

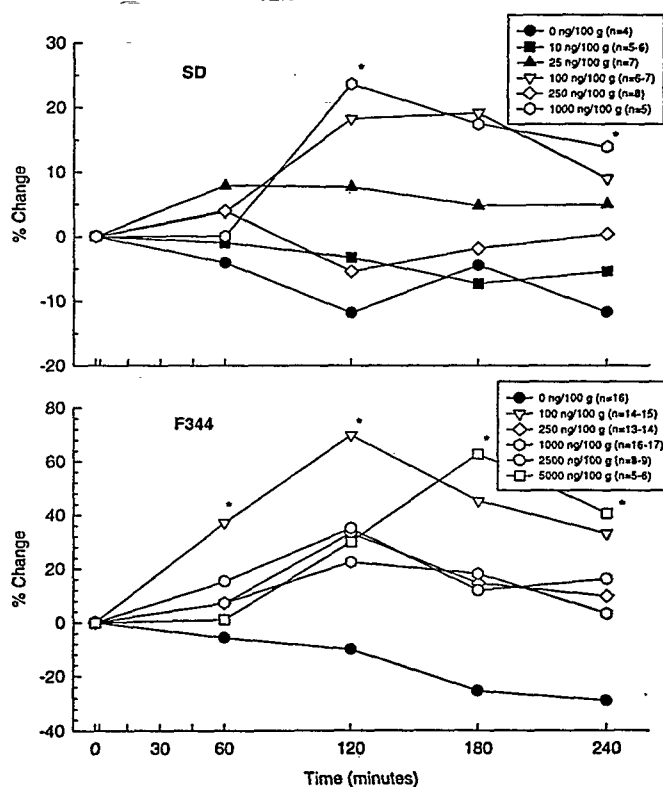


FIG. 3. Percent change in plasma T4 after TRH challenge in adult male SD and F344 rats. For T4 in both strains of rat, the standard error of the mean ranged from 5 to 20% of the mean for all time points across all dose groups. For most means, the SEM was $< 10\%$. Repeated measures ANOVA detected a significance due to effect of time, dose, and a time by dose interaction for the F344 rats, and an effect of time only for the SD. * $p < 0.05$ ANOVA or Mann-Whitney U.

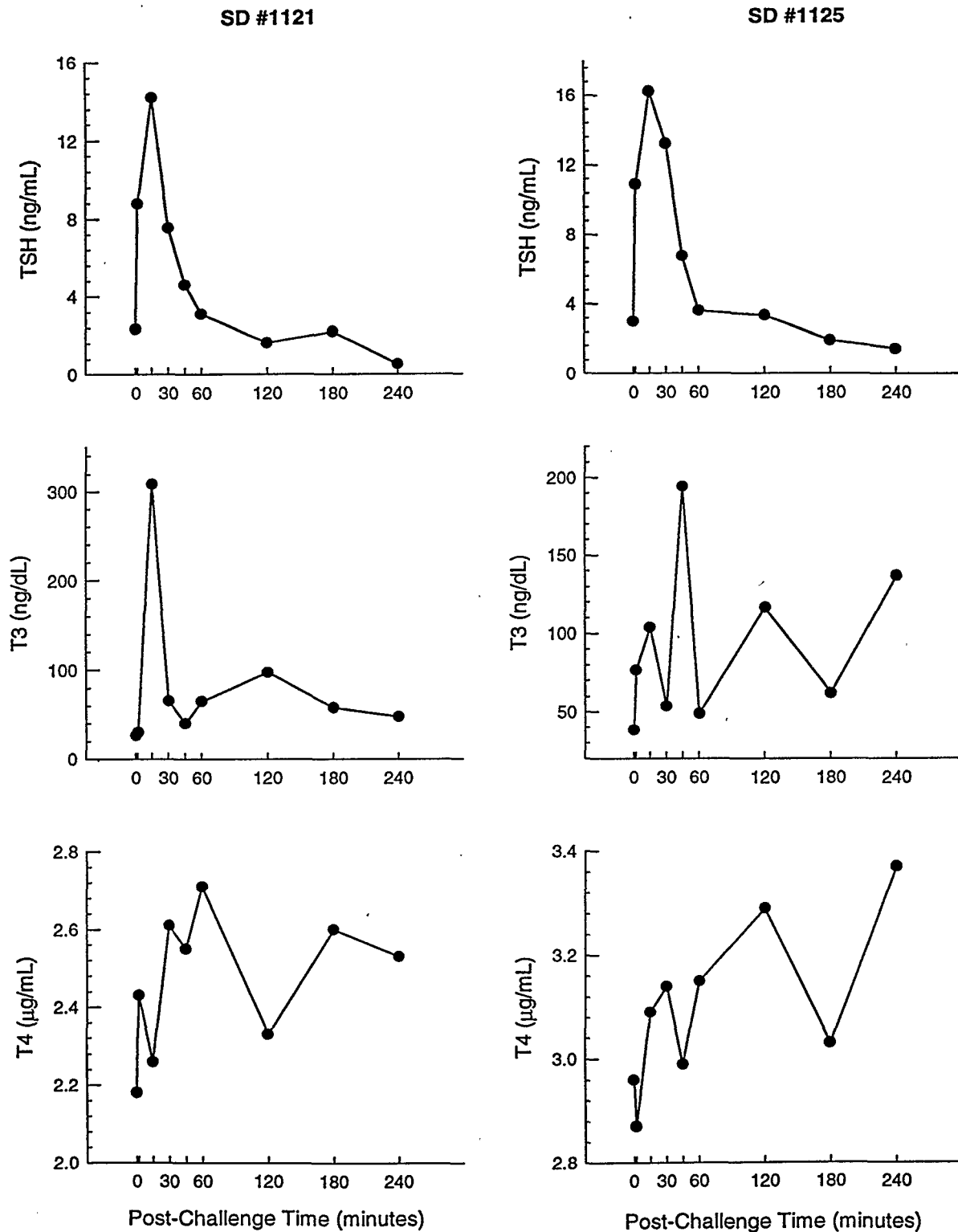


FIG. 4. Individual animal profiles for TSH, T3, and T4 after TRH challenge in adult male SD rats. Male SD rats (replicate 1) were selected from the 250-ng TRH/100 g body weight dose group (SD 1121 and SD 1125).

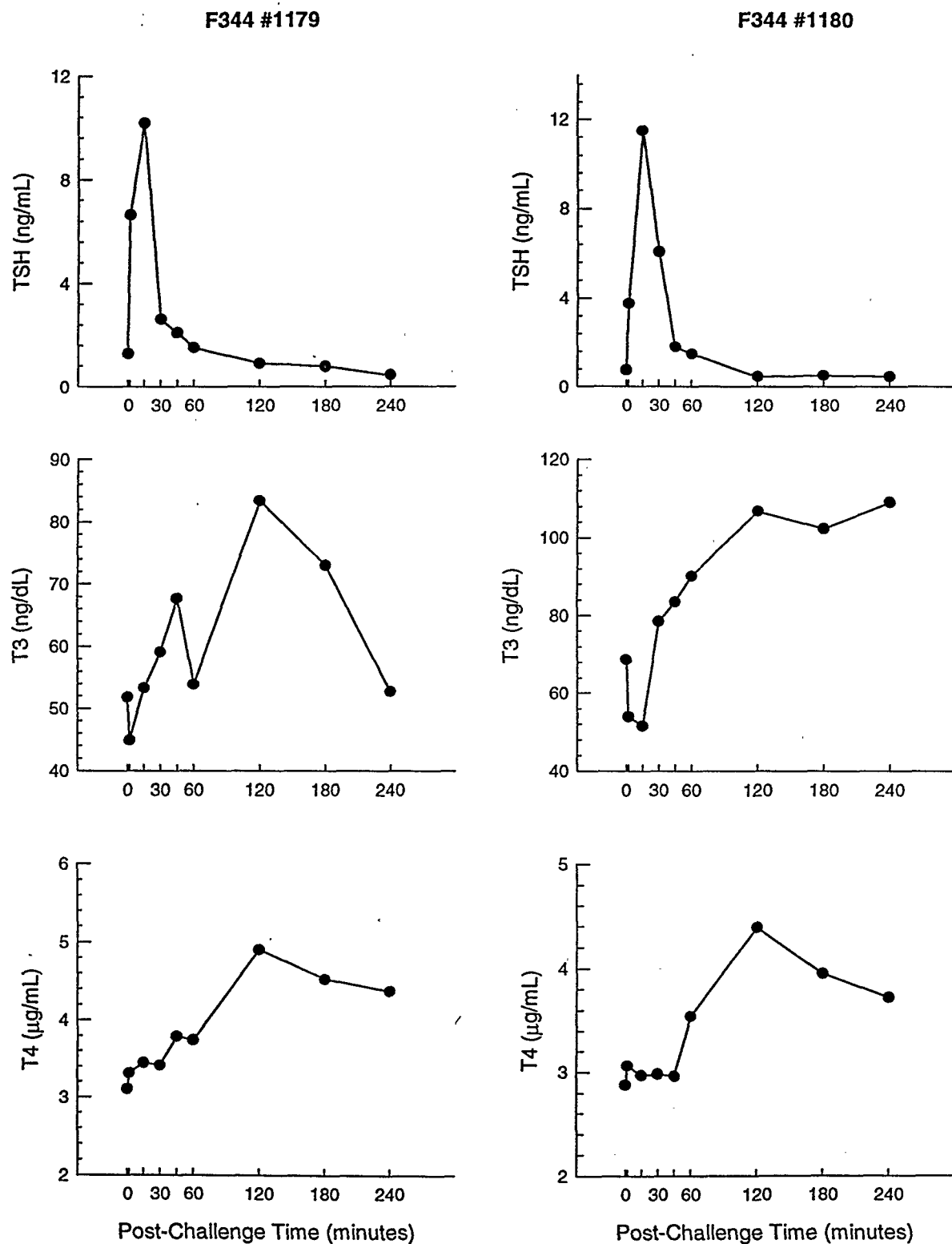


FIG. 5. Individual animal profiles for TSH, T3, and T4 after TRH challenge in adult male F344 rats. Male F344 rats (replicate I) were selected from the 250-ng TRH/100 g body weight (F344 1179 and F344 1180).

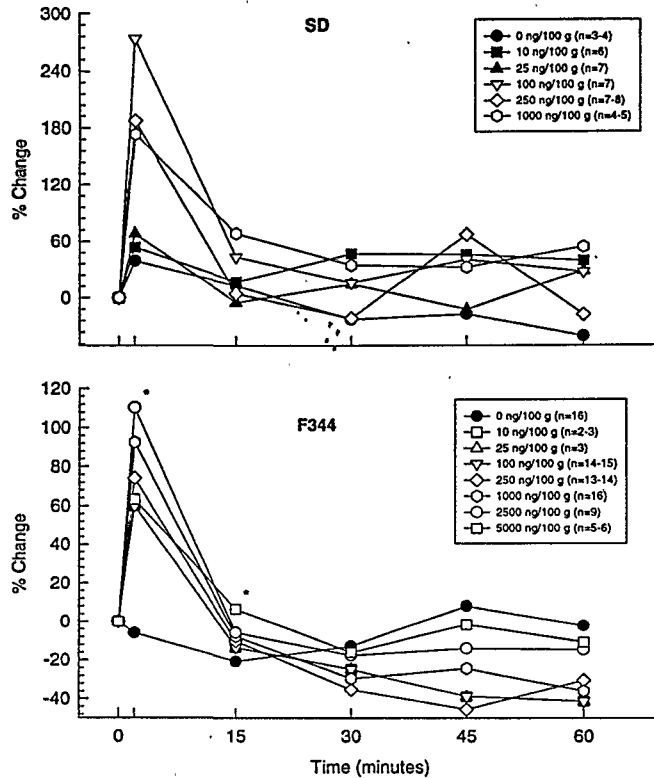


FIG. 6. Percent change in plasma prolactin after TRH challenge in adult male SD and F344 rats. For prolactin in F344, the standard error of the mean ranged from approximately 10–30% of the mean for all time points across all dose groups. For most means, the SEM was about 10%. Mean values ranges from 1.4 to 8.3 ng/mL in F344 male rats. Repeated measures ANOVA detected a significance due to effect of time, dose, and a time by dose interaction for the F344 rats, and an effect of time only for the SD rats. * $p < 0.05$ ANOVA or Mann-Whitney U.

they occurred spuriously within animal and one occurred even before treatment (data not shown). Inspection of individual profiles for prolactin indicates that the animals may have been stressed during the 30-min pretreatment period. This was evi-

dent from the decreasing profiles, not only for controls but also for all other groups. Rats were jacketed at 30 min before the first baseline sample.

Prolactin response to TRH in male F344 rats. The prolactin response to TRH is apparent using mean percent change, and the prolactin release after TRH in F344 rats was clearly dose dependent (Fig. 6) between 100 and 2500 ng/100 g body weight. A significant TRH-induced spike of prolactin occurred at 2 min after TRH at 250–2500 ng/100 g body weight, based on mean concentrations. For the 5000-ng TRH dose, the initial spike was not as large as the 2500-ng group (as would be expected in a dose response). The 5000-ng TRH dose group also had a significant increase in prolactin at 180 min (data not shown).

Hormone relationships in SD rats. The relationships of TSH, T3, and T4 to each other and to prolactin was examined using Pearson correlation coefficients. The relationship between pairs of hormones were examined at time zero (Table 4). Before TRH was infused, T4 concentrations were highly correlated only to prolactin concentrations ($p = 0.0144$). No other correlations were significant.

We also examined the correlation of the concentration of hormones (prolactin, TSH, T3, and T4) at time zero on the response of these hormones to TRH (Table 5). Only the concentration of T4 at time zero had any significant correlation to the T4 response to TRH. This correlation was positive, i.e., the lower the T4 concentration at time zero, the lower the response at 1 and 2 h ($p < .05$).

Hormone relationship in male F344 rats. In F344 male rats, T3 and T4 values were highly correlated within time zero ($p < 0.0001$) across animals in all dose groups ($n = 84$) (Table 4). No other significant correlations were observed.

The response of T3 and T4 to TRH at 1 and 2 h was highly dependent upon T3 and T4 concentration at time zero. The correlations were positive for both the 1- and 2-h time points. Both hormones were more highly correlated with their own concentration at time zero than with those of the other hor-

TABLE 4
Within Time Zero Correlations of Plasma Hormones in Adult Male Sprague-Dawley Rats and Fischer 344 Rats

Rat strain	Hormone pair	r	p	n^*
SD	T3 with T4	0.27382	0.0962	38
	T3 with TSH	0.14991	0.3690	38
	T4 with TSH	0.25423	0.1235	38
	T4 with Prolactin	0.39408	0.0144	38
F344	T3 with T4	0.47514	0.0001	84
	T3 with TSH	0.02538	0.8221	81
	T4 with TSH	0.09965	0.3731	82
	T4 with Prolactin	0.11831	0.2898	82

Note. r , Pearson correlation coefficient; p , significance level; n , number of paired data points.

* $n = 36 - 38$ per pair for SD and 81–84 for F344.

TABLE 5
Correlations of Plasma Hormones in Adult Male SD and F344 Rats after TRH ECT

Hormone at time zero	TSH @ 15 min	T3 @ 1 h	T3 @ 2	T4 @ 1 h	T4 @ 2 h
SD					
T3	-0.02767	0.29745	0.03663	0.08134	0.19519
T4	-0.09395	-0.02465	-0.12080	0.77498**	0.53406*
TSH	0.02793	0.16952	-0.11277	0.18366	-0.07026
PRL	-0.11629	0.11180	-0.14651	0.09358	0.10779
F344					
T3	0.23304	0.63624**	0.75206**	0.53294**	0.52043**
T4	0.16836	0.33454*	0.30167*	0.72773**	0.62942
TSH	0.13549	-0.04880	-0.07463	0.10179	0.06580
PRL	0.11031	0.08124	0.20698	0.11972	0.18193

Note. Numbers are *r*, Pearson correlation coefficient, for hormone at time zero with the hormone value at other times indicated after TRH ECT. Hormone sets (e.g., T3 at time zero with TSH at 15 min) were considered within animal strain for all doses combined. *n* = 81–84 per hormone set for F344 rats and 36–38 per set for SD rats.

* *p* < .001.

** *p* < 0.0001.

mones. The TSH and prolactin responses were not related to their own or to T3 or T4 concentration at time zero.

Experiment 2: Endocrine Response to Exogenous TSH Challenge

Thyroid response to TSH in adult male SD rats. The percent change from zero values were increased for the high-dose group by 60 and 120 min (Fig. 7). The area under the T4 curve (–30 to 240 min) was also significantly larger for this group of animals (areas were -12.80 ± 65.42 , 116.55 ± 7.80 , 48.65 ± 61.91 , and 221.25 ± 29.90 for 0, 200, 400, and 800 ng TSH/100 g body weight, respectively). Thus, to detect a significant increase in T4 after TSH dosing, a dose of at least 800 ng/100 g body weight would be needed. Sufficient TSH was not available to test higher concentrations. However, the plasma T3 concentrations (Fig. 8) were not significantly different from controls at any time point, due to low *n* and higher variation for T3 than for T4.

Thyroid response to TSH in adult male F344 rats. After the TSH ECT, mean concentrations of T3 and T4 were not significantly different from controls at any time (data not shown). However, calculations of percent response from the time zero samples clarified the response to TSH. At 60 min, plasma T4 was significantly increased after 1600 ng TSH/100 g body weight and continued to increase through the 240-min sample (Fig. 7). The T3 response was also significant at 120–240 min for this dose of TSH (Fig. 8). Neither the T3 nor the T4 response was completed by 240 min after TSH, so the maximum concentrations may have occurred later. Thus, to detect a significant increase in T3 or T4 after TSH dosing in F344 rats, more than 400 ng TSH and possibly as much as 1600 ng TSH/100 g body weight was needed. Because we were unable to test other dosage amounts of TSH, the ED₅₀ of TSH for T3 and T4 release was not identified.

Prolactin response to TSH in adult male rats. Prolactin concentrations were not increased at any time after TSH treatment (data not shown) in either SD or F344 rats.

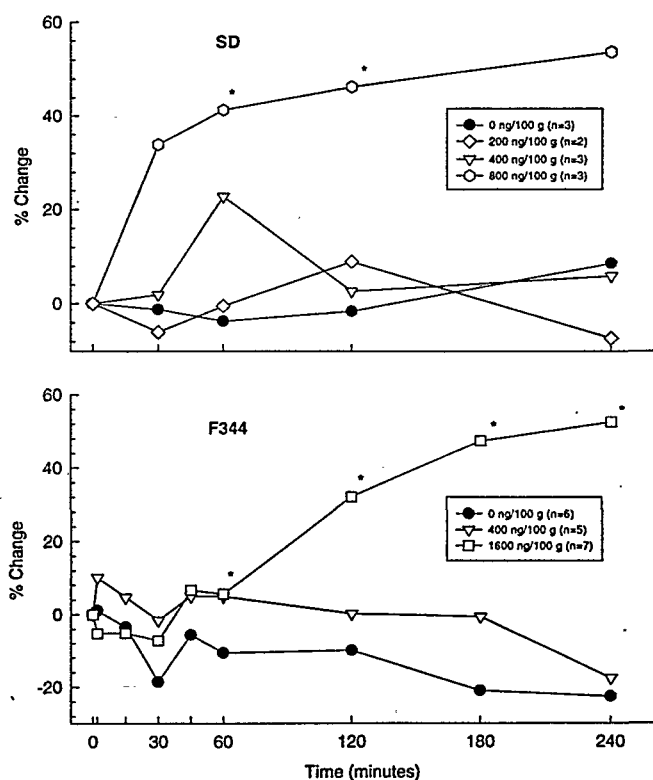


FIG. 7. Percent change in plasma T4 after TSH challenge in adult male SD and F344 rats. Repeated measures ANOVA detected a significance due to effect of time and a time by dose interaction for the F344 rats, and an effect of time only for the SD rats. **p* < 0.05 ANOVA or Mann-Whitney U.

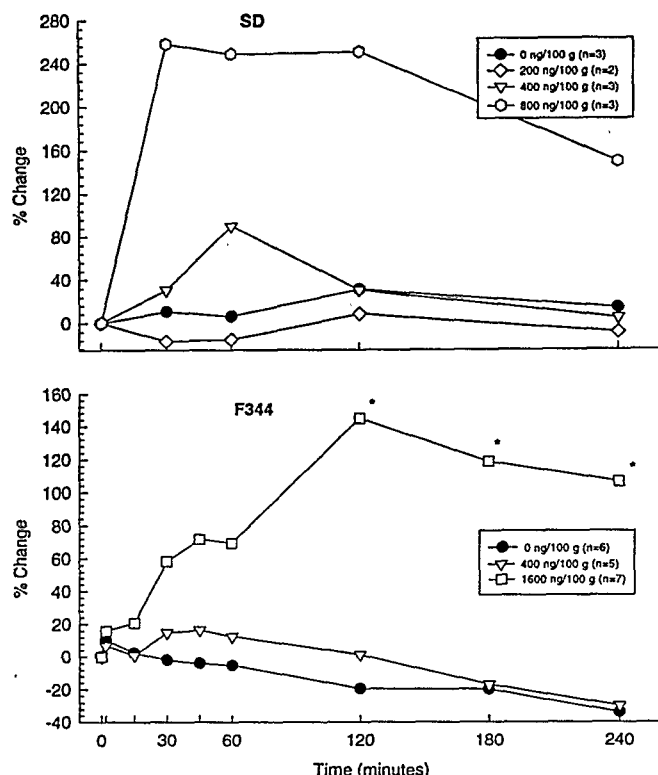


FIG. 8. Percent change in plasma T3 after TSH challenge in adult male SD and F344 rats. Repeated measures ANOVA detected a significance due to effect of time and a time by dose interaction for the F344 rats, and an effect of time only for the SD rats. * $p < 0.05$ ANOVA or Mann-Whitney U.

DISCUSSION

Endocrine Challenge Tests

Based upon the knowledge that TSH is elevated in many cases when thyroid hyperplasia and tumors are present, TRH and TSH ECTs could be used to define associated abnormalities in the HHTA. ECTs can define both the endocrine organ's magnitude of response and the pattern of hormone release over time. However, these responses will vary with the dose of secretagogue or tropin. Theoretically, after a TRH challenge some threshold dose will evoke a marginal release of TSH. The smallest dose of TRH causing marginal TSH release will be termed the excitatory dose (ED_{10}). That dose of TRH causing maximal release of TSH would then be called ED_{100} . (Higher doses of TRH would not evoke further TSH release.) The ED_{50} is that dose of secretagogue (TRH) causing release of target hormones (TSH) at a concentration that is one-half of the maximum releasable concentration. Excitatory or effective doses (ED) to be used in ECTs must be on the slope of the dose-response curve (e.g., ED_{50} or ED_{75}). Although supra-physiologic doses (i.e., $\geq ED_{100}$) can identify altered maximum response of endocrine organs, they are incapable of detecting changes in sensitivity or altered threshold (i.e., a shift of the

dose response to the right or the left without a change in maximal response) (Genuth, 1983). Use of the ED_{50} or ED_{75} in ECTs allows for detection of both altered sensitivity and altered responsiveness of an endocrine organ, maximizing the chance of identifying endocrine disruption. Thus, it is critical that the dose-response curve be characterized before using an ECT for diagnosis or experimental purposes. We have identified the ED_{50} of TRH in these experiments (Fig. 9). The dose response of pituitary and thyroid hormones after TRH are plotted for selected times (Fig. 10) and the ED_{50} of TRH for TSH release is calculated in Figure 9.

The TSH response to TRH. It was expected that for each hormone measured after ECT, a characteristic response profile would be observed. In this ECT, sampling times were selected to maximize detection of pituitary hormone release (TSH or prolactin) expected at 2 and 15 min. The time response of TSH after TRH in both strains of rat was as predicted, with maximal concentrations occurring at 15 min. Those responses are plotted for both rat strains in Figure 9, and the ED_{50} and the ED_{75} are calculated.

In F344 rats, the TSH response to TRH occurred, but was not significantly different between the higher dose groups

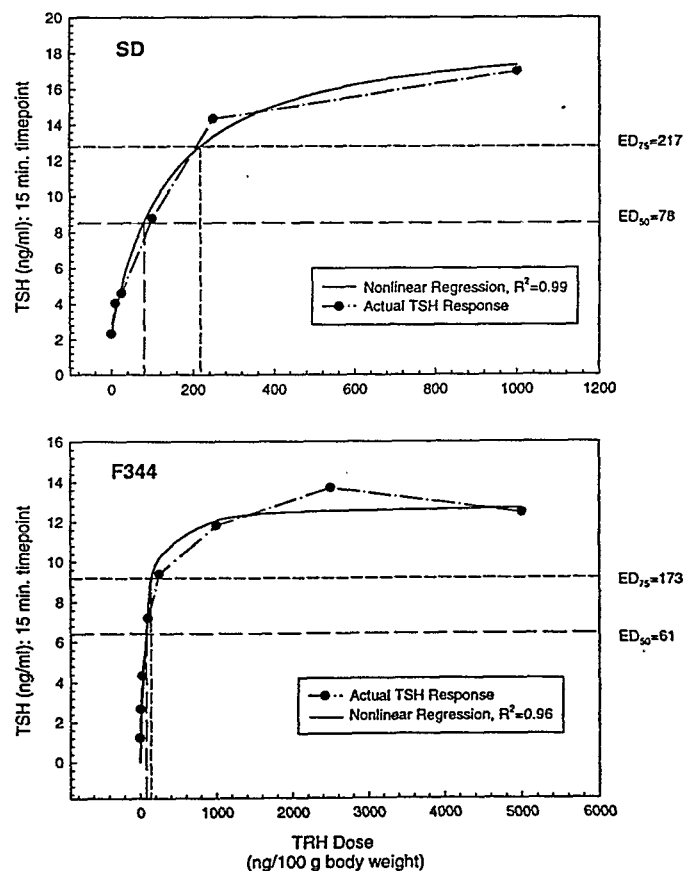


FIG. 9. TSH dose response to TRH challenge in adult male F344 and SD Rats: 15-min time point ED_{50} and ED_{75} .

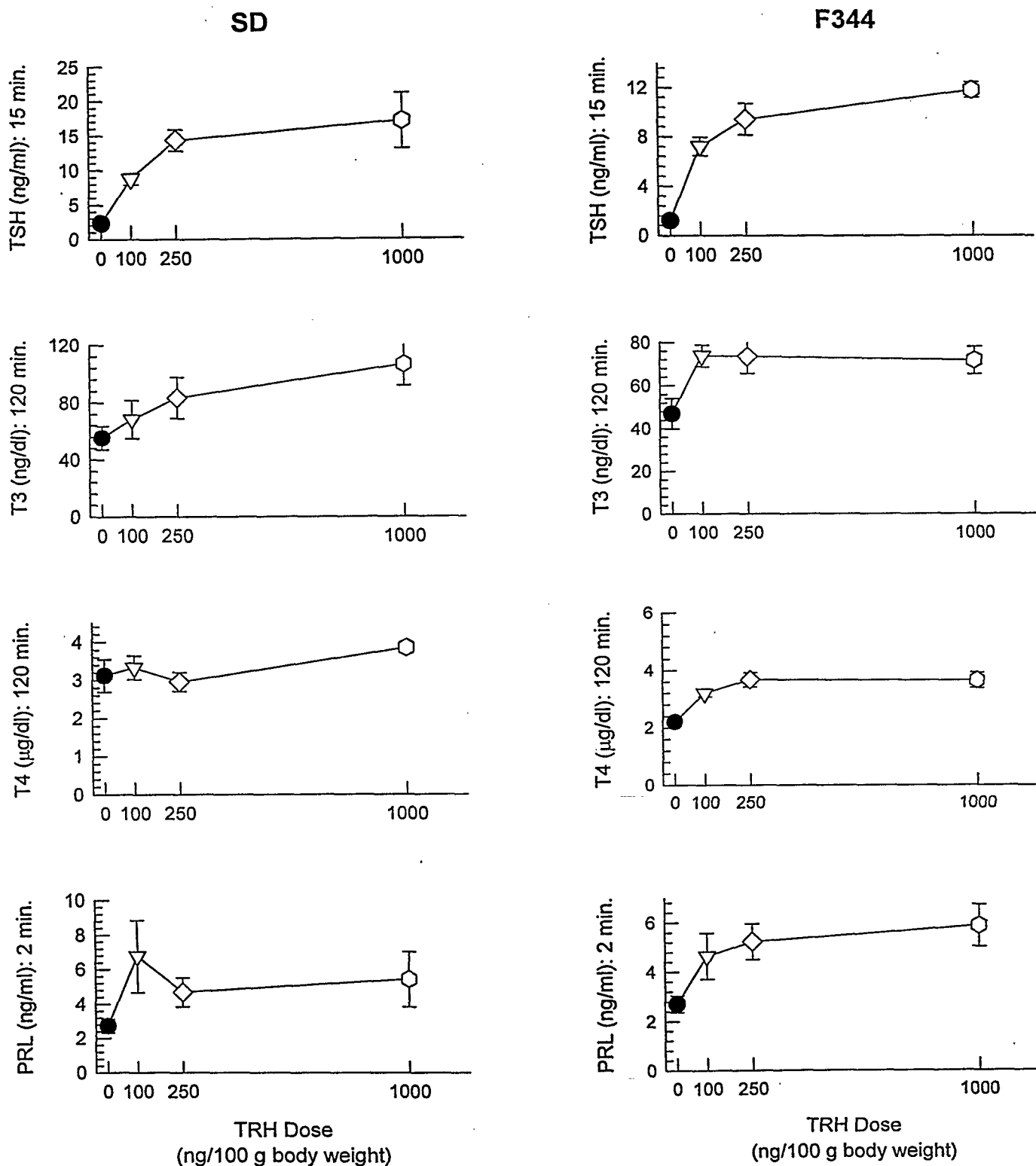


FIG. 10. Hormonal responses in HHTA after 100, 250, and 1000 ng TRH/100 g body weight: selected time points.

(1000, 2500, and 5000 ng/100 g body weight) at 15 min (maximum response time) (Fig. 1), implying that the maximal response (ED_{100}) had been achieved by all of these doses (Figs. 9 and 10). For SD rats, there was no statistical significance between TSH values for 250 and 1000 ng/100 g body weight at 15 min (maximum response time for ≥ 25 -ng dose groups), implying an ED_{100} may have been reached (Figs. 9 and 10).

The calculated ED_{50} of TRH for TSH release was 78 ng/100 g body weight for SD male rats and 61 ng/100 g for F344 male rats; ED_{75} values were 217 and 173 ng/100 g body weight for SD and F344 rats, respectively (Fig. 9). Based on the discussion alone, when designing endocrine challenge tests, the TSH response could be tested using 200 ng/100 g body weight in both rat strains.

The TSH pretreatment baseline suggests that TSH concentrations remained relatively steady before TRH in both SD and F344 male rats. After TRH (10 and 25 ng/100 g body weight) in both SD and F344 males, TSH release was maximal at 2 min and returned to baseline by 45 min. Therefore, the TSH response to TRH was similar in both strains.

TSH concentrations before and after TRH were higher and more variable in SD than in F344 rats. Doses of 1000 ng TRH/100 g body weight and higher caused maximal response at 15 min, and TSH returned to baseline by 60 min. At dose levels ≥ 250 ng TSH/100 g body weight, TSH concentrations were not as high in F344 rats as in SD rats at the 2-min or 15-min sample following TRH. Similarly, baseline values in most F344 males were slightly lower than in SD rats.

Nonetheless, percent changes are similar between strains at higher doses. Looking at percent change from the baseline (0-min sample) to the 15-min sample, F344 males had comparable values to the SD rats. However, at lower doses (10–250 ng TRH/100 g body weight doses), F344 males had a larger percent change than the SD rats (percent data not shown).

The thyroid hormone response after TRH. It was expected that T3 and T4 would increase over the 4-h period after TRH (induced by TSH release), beginning at 1 to 2 h, with T4 increasing before T3. In the F344 rats, the T3 and T4 response was as expected. The area under the T3 curve was larger for TRH-dosed animals (≥ 100 ng/100 g) than for controls. The same was true for T4 (≥ 25 ng/100 g body weight). No ED_{50} was calculated for T3 or T4 for either SD or F344 rats. More dose levels, longer sampling times, and larger groups sizes are needed to obtain sufficient information to make this estimate.

The T4 concentrations in F344 rats were less variable than those of T3. In spite of that variability, significant percent change in T3 concentrations (within animal) appeared earlier (at 45 min; data not shown) than changes in mean T4 (60 min). Significantly increased changes in T4 lasted longer than did changes in T3 (compare Figs. 2 and 3). The pattern was clearly not as expected, and responses in individuals varied.

The T3/T4 relationships (ratio) also differed by strain. Both

strains of rat had increased T4 over a period of time, beginning 1–4 h after TRH, based on the percent average response to TRH. However, T3 increased in parallel to T4 for only the F344 rats. Thus T3/T4 ratios in the two strains differ.

For T4, F344 rats had a larger percent change (Fig. 3) than did SD rats. This is true whether individual, dose group, percent change, or the duration of the response is considered. For T3, the opposite may be true. Overall, SD rats had a higher percentage response (Fig. 4). Most likely, the lack of significant differences in T4 for SD rats is due to a smaller number ($n = 4$ to 8) and larger intra-animal and/or interanimal variation for SD rats.

Individual profiles of T3 and T4 support strain difference in release patterns and relative T3/T4 ratio. The response to 250-ng TRH is presented for two SD and two F344 rats (Figs. 4 and 5). In animal SD1121 (Fig. 4), T3 spiked at 2 min, reaching values of > 300 ng/dl, followed by a sustained release of T4. In SD1125, T3 and T4 increased simultaneously, although T3 was not a steady release like that for T4. In F344 rats, both T3 and T4 were released over a period of hours (Fig. 5). T4 at maximum concentration values were higher in F344 rats than in SD rats, whereas T3 concentrations at maximum were higher in SD male rats.

The strain difference in T3/T4 response is further underlined by the calculated correlations (Tables 4 and 5). T3 and T4 response to TRH, particularly in F344 males, was highly dependent upon T3 or T4 concentration at the time of TRH treatment.

The strain difference in T3 and T4 increase after TSH may be due to differences in thyroid physiology. In some species a small percent of total thyroid response is T3, with additional T3 being produced in peripheral tissues (see introduction). On average, the response for T3 and T4 after TRH in F344 rats can be expected at 2 h, with concentration of T3 and T4 increasing in parallel. The percent response at 120 min was similar in both hormones (Figs. 2, 3, and 5). However, in SD rats the percent response in the T3 and T4 at 120 min were not similar (Figs. 2, 3, and 4).

The thyroid hormone response after TRH compared to other species. The T4 response of F344 and SD rats after TRH (Fig. 3) was similar to that of other animals. In horses, T4 was increased by 60 min and remained so over a period of 3 h after TRH (Fail, 1975; Hafs, 1976). Plasma T3 also increased in F344 male rats. This response in F344 rats was in contrast to the lack of a significant T3 response after TRH in SD rats (Figs. 2 and 3). In SD rats, a significant thyroid hormone response was not detected by our hourly sampling regimen. A higher variation was present in the SD rats and fewer animals were sampled.

The prolactin dose response to TRH. Maximal prolactin response to TRH occurred at 2 min after TRH. Concentrations returned to baseline between 15 and 30 min after TRH challenge. The dose response suggests a treatment-related release

of prolactin immediately after TRH for both SD and F344 rats at 2 min. The mechanism of the blunted prolactin response to 5000 ng TRH/100 g body weight in F344 rats is currently unclear. This group also had a second increase of the TRH. A similar relationship (smaller response at higher doses) also occurred in SD rats, though at a lower dose (1000 ng TRH). This phenomenon is likely related to animal variability. Perhaps the true dose-response curve is not linear. However, further experimentation is required to substantiate this possibility.

During a TRH ECT in pregnant female SD rats, prolactin increased in a biphasic manner after TRH. After a dose of 100 ng TRH, the first prolactin spike occurred at 2 min and another occurred at 2 h. After a larger dose (100 μ g TRH), the spikes occurred at 2 min and 1 h (Fail, P. A., personal communication). It is possible that higher doses of TRH evoked a biphasic release of prolactin.

T3 and T4 response after exogenous TSH ECT. In the TSH ECT, T3 and T4 were increased after the higher doses of exogenous TSH as after TRH (endogenous TSH release). However, the T3 response was not significant in SD male rats. Therefore, in design of a TSH ECT, ≥ 800 ng TSH/100g body weight would be required to cause a T3 and T4 release.

Methods Control for Stress-Induced Hormone Changes

The studies reported here relied on cannulation of freely moving rats. To accomplish this, the animals were fitted with jackets to hold the cannulae in place. Animals were acclimated to jackets periodically for 3 days before the TRH ECT was instituted. On the day of the experiment, Sprague-Dawley rats were jacketed at 30 min before the initial sample. Based on prolactin data from experiments with SD males (recall that decreasing baselines were observed), it appears that rats need to be dressed in jackets at least 60 min prior to sampling to allow recovery from stress-induced prolactin release. In F344 males, animals were allowed over 60 min from the time jackets were put on until the first sample was collected. Again, we observed a decreasing baseline (i.e., prolactin concentrations were highest in the earliest samples; data not shown). Nonetheless, TSH baselines were steady. This downward drift in baseline prolactin cannot be treatment related (ECT dose). It is well known that 30 min are required for prolactin to return to baseline after a stressor, and it appears to have taken longer in this case (jacketing animals and attaching tethers to ports). Alternatively, the decreasing baseline could be associated with collection of the first sample (entrance of the technical staff into the rooms) or with a diurnal rhythm. However, neither of these events caused maximal prolactin release; when the 24-h sample was collected from the SD rats using manual restraint of the rat, prolactin levels were 1.5–4 times higher than the values seen at the 30-min time point (data not shown). At no time during these presampling periods for SD or F344 rats did

TSH concentrations appear to be affected by stress, because baseline concentrations of TSH remained constant.

Summary and Significance

This study compared the response of F344 and SD rats to a TRH ECT and a TSH ECT and characterized the dose-response curve. TSH, T4, T3, and prolactin responses were characterized for several doses of TRH over a 4-h time period in animals equipped with intra-atrial cannulae. Both strains of rats responded to intravenous TRH by releasing TSH. Concentrations reached a maximum at 15 min. The ED_{50} of TRH was 61 ng in F344 rats and 78 ng in SD rats. The ED_{75} s were 173 ng and 217 ng/100 g body weight, respectively. The response of T4 and T3 after TRH ECT and TSH ECT was highly variable. F344 rats responded with an increase in levels of both hormones, starting at 60 min and continuing through 240 min. In SD rats, a T4 response was present, but the T3 response was not detected. These data provide essential information for design of toxicology studies focused on the effects of toxicants and drugs on the pituitary-thyroid axis.

Further Work on Endocrine Challenge Test Dose-Response Data

The TRH ECT. These data are an important initial step in defining the dose-response curves in the HHTA of rats. Further testing is required. For SD rats, higher doses of TRH should be tested to verify maximal TSH release. It also seems important to test higher doses of TRH to more completely define prolactin response to TRH. In addition, a longer training and pretest period is required for rats to acclimate to their jackets. After acclimation, the first baseline sample should be taken, and a steady state should occur before the first ECT is done. Thus, a longer baseline period may be required before each ECT. However, unless prolactin is the hormone of interest, this seems unnecessary.

The TSH ECT. It also seems important to test higher doses of TSH and sample more frequently after TSH in a larger number of male rats. The T3 and T4 response after exogenous TSH seems to be less variable than after TRH-induced TSH release. These differences may be due to more accurate timing of the actual TSH release into the blood and less metabolism of TSH before it binds to TSH receptors.

Better estimation of T3, T4 response. Further characterization of the relationship of T3 and T4 to a TSH stimulus (for both rat strains) would aid in timing sample collection. Concentrations of TSH, T3, and T4 every 15 min after TRH should be completed. The data would reveal whether T3 and/or T4 increase in a broad surge after TSH release, do not respond, or are released in a pulsatile fashion. Where T3 and T4 response are of interest in females, the actual changes after TRH should be characterized before designing single-dose ECTs.

Designing single-point endocrine challenge tests. Thus, TSH concentrations are less variable (and more predictable) after TRH than those of T3 and T4. The design of a one-point TRH ECT for TSH response would be feasible. Animals could be injected (with an intravenous ED₅₀ to ED₇₅ of TRH) at time zero and one sample collected at 15 min after treatment. The TRH dose and blood sample collection time would differ with route of administration, strain, sex, age, and species.

ACKNOWLEDGMENTS

The evaluation of responses to TRH and TSH endocrine challenge tests in male SD and F344 rats was conducted at Research Triangle Institute under contract to Cytec Industries, Inc. The authors gratefully acknowledge the RTI research team for their technical assistance. Endocrine toxicology personnel included Susan Pearce, Betty McTaggart, and Barbara Gibson. Secretarial and clerical assistance was provided by Melinda Kantor, Carol Bailey, and Pam Leathers. Animal husbandry and health care was provided by Donald Feldman, Frank Ali, and technical staff of the Animal Research Facility.

REFERENCES

- Brown, C. E., Warren, S., Chute, R. N., Ryan, K. J., and Todd, R. B. (1979). Hormonally-induced tumors of the reproductive system of parabiosed male rats. *Cancer Res.* 39, 3971-3976.
- Buckingham, J. C., Gillies, G. E. (1990). Hypothalamus and pituitary gland—xenobiotic induced toxicity and models for its investigation. In *Endocrine Toxicology*, Chapter 4, pp. 85-114. Cambridge University Press, Cambridge.
- Chapin, R. E., Gulati, D. K., Fail, P. A., Hope, E., Russell, S. R., Heindel, J. J., George, J. D., Grizzle, T. B., and Teague, J. L. (1993). The effects of feed restriction on reproductive function in Swiss CD-1 mice. *Fundam. Appl. Toxicol.* 20, 15-22.
- Fail, P. A. (1975). (located under Noden, Patricia Ann). Estrus, ovulation, and changes in some Hormones during the estrus cycle of mares and after prostaglandin F2 α . Ph.D. Dissertation, Michigan State University, East Lansing, MI.
- Genuth, M. (1983). Section IX: The endocrine system. In *Physiology* (Berne and Leug, Eds.), Chapter 49, pp. 895-914. C.V. Mosby Co., St. Louis.
- Hafs, H. D. (1976). Some assay restrictions in inferences made from determining hormones in horses, cows, and their fetuses. *J. Toxicol. Environ. Health* 1, 669-679.
- Hennen, G. (1994). Cancer of the thyroid—explorations of clinical biology. *Acta. Chir. Belg.* 94, 30-32.
- Hill, R. N., Erdeich, L. S., Paynter, O. E., Roberts P. A., Rosenthal S. L., and Wilkinson, C. F. (1989). Thyroid follicular cell carcinogenesis. *Fundam. Appl. Toxicol.* 12, 629-697.
- McClain, R. M. (1992). Thyroid gland neoplasia: non-genotoxic mechanisms. *Toxicol Lett* 64/65, 397-408.
- Meites, J., Cassell, E., Clark, J. (1971). Estrogen inhibition of mammary tumor growth in rats; counteraction by prolactin. *Proc. Soc. Exp. Biol. Med.* 137, 1225-1227.
- Neumann, F. (1991). Early indicators for carcinogenesis in sex-hormone-sensitive organs. *Mutat Res.* 248, 341-356.
- SAS Institute Inc. (1989a). *SAS® Language and Procedures: Usage*, Version 6, First Edition, SAS Institute Inc., Cary, NC, 638 pp.
- SAS Institute Inc. (1989b). *SAS/STAT® Users' Guide*, Version 6, Fourth Edition, Volumes 1 and 2, SAS Institute Inc., Cary, NC, 1686 pp.
- SAS Institute Inc. (1990a). *SAS® Language: Reference*, Version 6, First Edition, SAS Institute Inc., Cary, NC, 1042 pp.
- SAS Institute Inc. (1990b). *SAS®: Procedures Guide*, Version 6, Third Edition, SAS Institute Inc., Cary, NC, 705 pp.
- SAS Institute Inc. (1990c). *SAS® Companion for the VMS® Environment*, Version 6, First Edition, SAS Institute Inc., Cary, NC, 458 pp.
- Sheppard, M. C. (1986). Serum thyroglobulin and thyroid cancer. *Q. J. Med.* 59(299), 429-433.
- Snedecor, G. W., and Cochran, W. G. (1967). *Statistical Methods*, Sixth Edition, Iowa State University Press, Ames, IA.
- Welsch, C.W., Jenkins, T.W., Meites, J. (1970). Increased Incidence of Mammary Tumors in the Female Rat Grafted with Multiple Pituitaries. *Cancer Res.* 30, 1024-1029.
- Williams, G.R. (1994). *Thyroid Hormone Regulation of Gene Expression*. R.G. Landes Company, Austin, TX.